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SPINAL SYMPATHETIC REFLEXES INITIATED BY CORONARY RECEPTORS

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SUMMARY

1. The main left coronary artery of vagotomized spinal cats was perfused at different flows and pressures. The changes in pressure were limited to the coronary bed.

2. Increased coronary flow which increased coronary arterial pressure provoked a reflex increase in sympathetic discharge in the white ramus of the third thoracic spinal nerve and the inferior cardiac nerve. Reflex reductions in activity were not observed.

3. Occlusion of the coronary sinus and myocardial ischaemia, due to cessation of pump inflow, evoked similar reflex increases of sympathetic activity. The effect of myocardial ischaemia was apparent before systemic arterial blood pressure fell or left ventricular end-diastolic pressure rose.

4. Increased coronary arterial pressure, myocardial ischaemia and coronary sinus occlusion could activate the same preganglionic neurone.

5. The afferent limb of the excitatory coronary-sympathetic reflex was in the cardiac sympathetic nerves, mainly on the left. Afferent nerve fibres running in these nerves and in the third left thoracic sympathetic ramus communicans were excited by increased coronary arterial pressure, myocardial ischaemia, and occlusion of the coronary sinus. Inhibition was not observed. Many of the receptors were further localized by direct probing over the coronary vessels and adjacent myocardium.

6. Some receptors were excited by increased coronary arterial pressure alone, others by coronary sinus occlusion, and still others by myocardial ischaemia. In addition, some receptors were excited by all three stimuli.

* All the experiments described in this paper were performed in the U.S.A.

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INTRODUCTION

In man, myocardial ischaemia produces pain which is abolished or greatly reduced by bilateral stellectomy and excision of the upper five thoracic sympathetic ganglia (Lindgren & Olivecrona, 1947). Brown (1967) demonstrated that transient occlusion of the main left coronary artery in cats provokes a pain-like or pseudaffective response (Sherrington, 1906) due to increased nervous discharge in afferent fibres running in cardiac sympathetic nerves. Using intact brain, decerebrate and spinal cats, Malliani, Schwartz & Zanchetti (1969) found that transient occlusion of a ramus of the left coronary artery can reflexly activate preganglionic sympathetic fibres in the third left thoracic ramus communicans. This nerve contributes importantly to the innervation of the heart (Bronk, Ferguson, Margaria & Solandt, 1936; Randall, McNally, Cowan, Caliguiri & Rohse, 1957). Thus, the evidence pointed to a coronary-sympathetic reflex mediated through the spinal cord although the stimulus used, namely, occlusion of a vessel, was imprecise since it was accompanied by an unknown amount of mechanical distortion. Brown (1964) had also found afferent fibres in cardiac sympathetic nerves that were stimulated by mechanical probing of the heart and similar findings were made by Ueda, Uchida & Kamisaka (1969). It was decided, therefore, to investigate the effects of changes in coronary flow and pressure on the discharge of efferent and afferent fibres in cardiac sympathetic nerves of spinal vagotomized cats. We found that both increased coronary flow which increased coronary arterial pressure, and coronary sinus occlusion, provoked a reflexly mediated *increase* of cardiac sympathetic discharge. Myocardial ischaemia had the same effect. The afferent limb of this excitatory coronary-sympathetic reflex was in the cardiac sympathetic nerves and the receptors were further localized by direct probing over the coronary vessels. Some receptors were excited by only one of the modalities of stimulation, whereas others were excited by all of the modalities used.

METHODS

Experiments in which there were no technical failures were done on twenty-five cats (2·5–5·0 kg) anaesthetized by the intraperitoneal injection of pentobarbitone (35 mg/kg). The trachea was cannulated and polyethylene catheters were inserted into a femoral vein and a femoral artery. In two experiments, catheters were inserted into both atria and both ventricles. Pressures were measured with Statham P 23 dB strain gauges and the measured flat (± 5 %) frequency responses were 20–30 c/s. Mean pressures were registered according to Brown (1968). The animals were paralysed with gallamine triethiodide (Flaxedil, Davis and Geck) 2 mg/kg and artificial ventilation begun. The stroke and rate of the respiratory pump were adjusted to maintain end-tidal P_{CO_2} measured with an infra-red CO_2 meter at

28 mm Hg which is approximately normal for cats in Salt Lake City (elev. 4200 ft). Arterial P_{CO_2} ranged from 24 to 30 mm Hg, arterial P_{O_2} 70 to 80 mm Hg and arterial pH 7·42.

The spinal cord was sectioned at the level of C1. Both vagi were cut, and in all experiments one common carotid artery was ligated. In two experiments the other carotid artery was also ligated. Neural recording was begun about 4 hr after spinal section. After the effects of gallamine had subsided, the neck and face were pinched periodically to elicit either a pain-like reaction, pupillary dilatation or changes in blood pressure. If any response occurred, supplemental doses of pentobarbitone ($\frac{1}{6}$–$\frac{1}{8}$ the initial dose) were given.

Coronary perfusion. The main left coronary artery was perfused according to the method of Brown (1968). The chest cavity was entered through the fourth left interspace. Blood was led from one common carotid artery to a pump (Holter Co.) and thence to a stainless-steel cannula. The cannula was passed down the left subclavian artery into the main left coronary artery where it was tied into place. Flow was set at 6–8 ml./min which is somewhat greater than the flow rate of 5 ml./min measured with a bubble flowmeter (Brown, 1964). The pressure in the inflow coronary arterial line was registered with a Statham strain gauge and was generally 10–15 mm Hg greater than arterial blood pressure. The animals were heparinized (1000 units I.V. initially and 500 u./hr later). Successful experiments lasted up to 6 hr without any deterioration of the animal's arterial blood pressure and heart rate.

Occlusive procedures. The coronary sinus was dissected carefully and a ligature placed loosely around it. The sinus was occluded by pulling the ligature and contained vessel against a piece of polyethylene tubing. Similar methods were used to occlude the aorta or main pulmonary artery.

Nerve recording. The method of exposing the left stellate ganglion and its branches has been described (Brown, 1967). Efferent recording was done from the cut central end of slips of nerve dissected from the sympathetic white ramus of the left third thoracic spinal nerve (T3), which innervates the heart (Bronk *et al.* 1936; Randall *et al.* 1957), or the left inferior cardiac nerve. Afferent recording was done using slips of nerve dissected from the cut peripheral end of the white ramus of T3 or the inferior cardiac nerve. The recording electrodes were platinum; they were connected to a.c. preamplifiers with a band width of 10 c/s–3 kc/s.

Filaments of nerve were progressively divided and the response of spontaneously active or previously quiescent fibres to increased or decreased coronary flow and in later experiments, coronary sinus occlusion, was tested following each division. Dissection was continued until ideally, a single, responsive unit was isolated, such a unit being distinguished by constant amplitude and configuration. Each receptive unit was studied for at least 15 min and often for 1–2 hr. Sometimes changes in amplitude of unitary activity occurred, probably due to changes in moisture content of the fine nerve strands. However, even with amplitude alterations, the configuration usually remained stable, a point which was repeatedly checked during the experiment and more importantly, after the experiment, using an impulse-sorting procedure to be described below. This procedure permitted identification of four to five different units in a multifibre record.

The action potentials, pressure records and electrocardiogram were recorded on tape (Ampex FR 1300). Preliminary analyses of the results were made by replaying the tape records onto an oscilloscope and a multichannel ultraviolet recorder (Honeywell, 1508). The impulse-sorting procedure and quantitative histograms based on plots of 'instantaneous' frequency (1/interval between successive impulses) were done using the interactive system described by Schmittroth in Bessou & Perl (1969). Briefly, the experimenter identifies a single unit, using shape and amplitude

as criteria, on a large digitally controlled oscilloscope having a digital memory and small computer to interface with the main computer (Univac 1108). The program then sorts impulses using Fourier series coefficient comparisons and a filter based on the maximum and minimum values. A finite Fourier sine series is computed for each impulse of a sample and the program determines the maximum and minimum coefficients for the first eight harmonics of the particular impulse. On command, the program proceeds through a sample of different impulses sequentially, comparing the shape of each impulse with that of the particular impulse of interest, selecting those which fit between the predetermined limits. The experimenter can check the accuracy of the computer by studying each impulse selected by the machine. After sorting the impulses the program then computes the time intervals between pulses and the 'instantaneous' frequency of each pulse. The frequency is then displayed against another time-varying parameter such as blood pressure.

RESULTS

Haemodynamic events associated with changes in coronary flow. Increases in coronary flow and pressure over the range of 4–20 ml./min for periods of 1–2 min have no effect on right atrial pressure, right and left ventricular pressures, and aortic pressure (Figs. 1 and 2). Heart rate, left atrial and pulmonary arterial pressures, although not shown in these Figures, were also unchanged. The coronary arterial pressure, however, showed stepwise increases with increases in flow. Therefore, the stimulus was restricted to the coronary bed and probably the adjacent myocardium.

Occlusion of the coronary sinus at normal inflows had either no effect on coronary arterial pressure or caused a small increase of 10 mm Hg or less. This implies that collateral venous channels can ordinarily accommodate the left coronary arterial inflow. However, changes would be expected in pressure or volume of venous channels upstream from the coronary sinus. The degree of ischaemia, if any, that may have been produced by sinus occlusion in these experiments is unknown. At higher inflows (15–20 ml./min) coronary sinus occlusion always increased coronary arterial pressure greater than 10 mm Hg (Fig. 10*B*).

Cessation of inflow which reduced myocardial oxygen tension (Brown, 1968) caused myocardial ischaemia by definition and a fall in coronary arterial pressure. The fall in coronary arterial or inflow pressure was variable depending upon the collateral circulation from the right coronary artery. In Fig. 1, inflow pressure fell to 40 mm Hg, whereas in other experiments it fell to practically zero mm Hg. The degree of ischaemia therefore probably depended upon the extent of collateral circulation. For example, the animal which gave the response seen in Fig. 1 showed no evidence of a fall in arterial blood pressure after 2 min ischaemia. After 3 min the pressure fell and after 5 min the animal was dead. In other cats, heart failure defined as a fall in aortic pressure and/or a rise in left ventricular end-diastolic pressure occurred after 40–60 sec.

Effects of increased coronary inflow on sympathetic preganglionic activity. The positive pressure phase of artificial respiration caused a small rise in arterial and venous pressures. Such changes in pressure are seen in Fig. 1 on a slow time base and in Fig. 2 on a faster time base. Equally small changes in coronary pressure produced by small increases in coronary flow had no effect on neural discharge recorded in the present experiments.

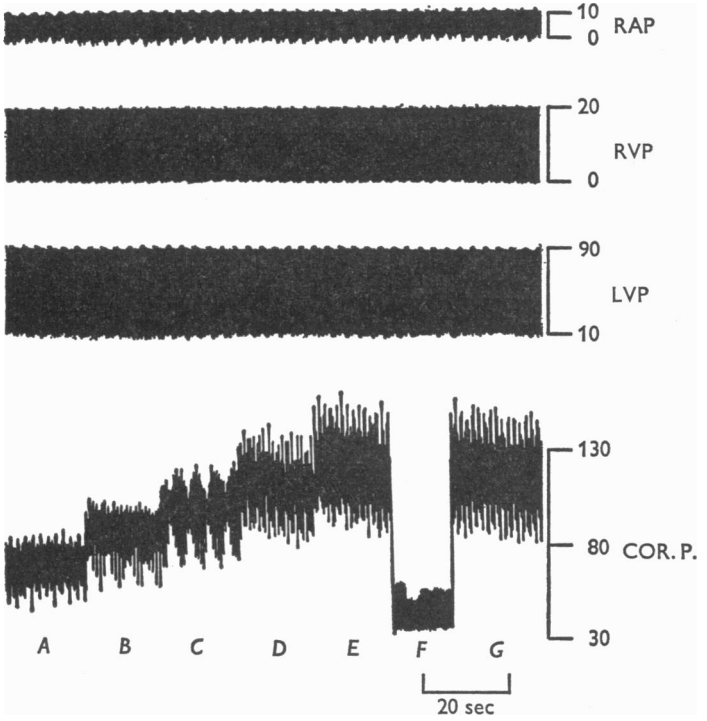

Fig. 1. Effect of different levels of coronary flow on coronary arterial or inflow pressure, left ventricular (LVP), right ventricular (RVP) (mm Hg) and right atrial (RAP) (cm H_2O) pressures. The flow was 4, 8, 12, 16, 20, 0 and 20 ml./min at *A–G*. There was no change in any of the pressures other than coronary arterial pressure. Left atrial, pulmonary arterial and aortic pressures were also unaffected. At zero inflow, the mean inflow pressure was 40 mm Hg, indicating good collateral flow from the right coronary artery. Pressure traces show fluctuations phasic with positive pressure respiration.

Larger increases of coronary inflow, which raised coronary arterial pressure substantially, provoked an increase of preganglionic discharge in nerve fibres in the white ramus of T3 in 15/15 experiments. Reflex reduction in sympathetic activity has not been observed. The positive responses were obtained in twelve multifibre preparations and eight single units. A summary of the number of single units and multifibre preparations

activated by the various stimuli used is given in Table 1. Fig. 2 shows the early response in a multifibre preparation when the inflow (previously set at zero for 1 sec) was raised abruptly from zero to 20 ml./min. The fibres were not excited by a 40 sec period of ischaemia alone. After 0·5 sec, a biphasic spike began to discharge, and after 2·0 sec, a tall monophasic spike also became active. When the activity of the biphasic spike was analysed by the computer, the record of Fig. 3*A* was obtained. This shows the response over another test period during which coronary arterial pressure was increased. The increased pressure shown as a downward

TABLE 1. Number of efferent fibres excited by stimuli limited to the coronary bed

	Increased coronary arterial pressure	Coronary sinus occlusion	Myocardial ischaemia
Single units	8	2	4
Number of responses	67	13	18
Success rate (%)	100	92	89
Multifibre preparations	12	1	4
Number of responses	48	6	22
Success rate (%)	100	100	97

The two single units and one multifibre preparation in column two were also excited by increased inflow and are included in column one. One of these single units was also stimulated by myocardial ischaemia and is included in all three columns. The remainder of the fibres in column three were excited by myocardial ischaemia alone.

deflexion evoked a burst of activity with a peak frequency of 23 impulses/sec in this fibre. The burst subsided quickly and activity ceased after 4–5 sec. The inset shows the biphasic spikes counted by the computer during this time, superimposed at faster sweep speed. It is clear that the activity of one single unit has been assessed. When the tip of the coronary arterial cannula was moved, a brief burst from this unit was also provoked (Fig. 3*B*).

In two other experiments, a burst of activity was provoked only at the initiation or cessation of the increase in coronary pressure (Fig. 4*A*) with activity practically absent during the maintained pressure increase. The unit shown in Fig. 4*A* fired occasionally before the pressure was changed. Again, manipulation of the tip of the cannula elicited a discharge (Fig. 4*B*). Three other single fibres (13/13 trials) and four multifibre preparations (15/15 trials) responded similarly to movement of the tip of the coronary cannula.

The fibres shown in Figs. 2, 3 and 4 did not have a sustained discharge with increased coronary pressure. However, three fibres were observed to

show progressively increasing activity when the coronary pressure was elevated. Such a fibre is illustrated in the histogram of Fig. 5. The spikes were counted during 2 sec periods and plotted against coronary pressure. Spontaneous discharge was present (Fig. 5*A*) and 15 sec after the pressure had been elevated the activity increased and continued to rise over the next 24 sec of sustained pressure increase (Fig. 5*B*). When the pressure was raised again, the discharge after a latency of 24 sec reached a new high level and remained there (Fig. 5*C*).

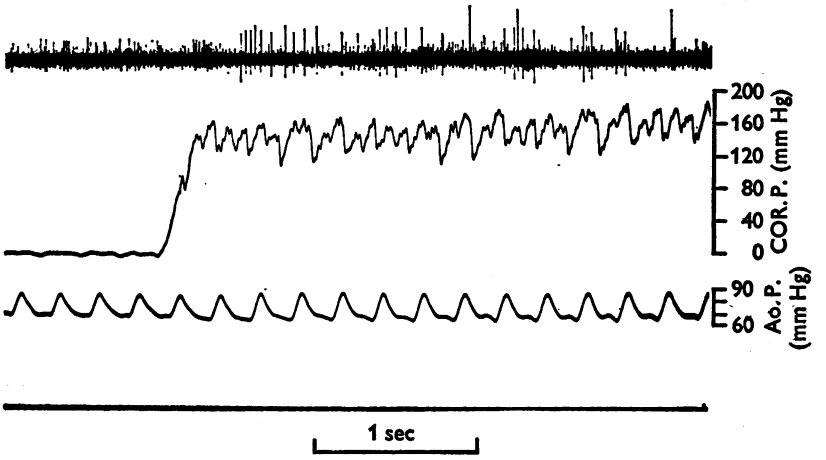

Fig. 2. Effect of increasing inflow from 0 to 20 ml./min on a few efferent fibres dissected from the third left white ramus (top or first trace). The mean inflow pressure rose from 0 to 160 mm Hg ((COR.P.) second trace). Aortic pressure (Ao. P.) showed small changes in shape related to positive pressure respiration and not to changes in coronary flow (third trace). The coronary arterial pressure had a complex pattern generated from the intramyocardial pressure, aortic pressure and inflow pump. Animals were spinal and vagotomized in this and all subsequent records unless otherwise indicated. The noise levels in the electroneurographic records ranged from 10 to 20 μV.

The reflex nature of the evoked discharge was clearly demonstrated in three experiments. The left stellate ganglion and all its branches were excised. In one experiment, the discharge was abolished and in the other two, it was greatly reduced. When the right stellate and its branches were then excised, the remaining evoked activity was abrogated. Afferent fibres in cardiac sympathetic nerves which are excited by increases in coronary inflow pressure were subsequently identified.

The reflexly evoked impulse activity was not altered by prior inhalation of 100% oxygen.

Other effective stimuli limited to the coronary vascular bed. Occlusion of the coronary sinus provoked a reflex increase in activity in two single units

and one multifibre preparation (Fig. 6*B* and Table 1). Inflow pressure was increased when the coronary sinus was occluded in the case of the unit shown in Fig. 6*B*, but a similar increase in activity occurred without an accompanying rise of inflow pressure in another single unit (5/5 trials). These fibres were subsequently excited by an increase in coronary inflow (Fig. 6*A*).

Zero pump inflow which caused myocardial ischaemia also provoked a

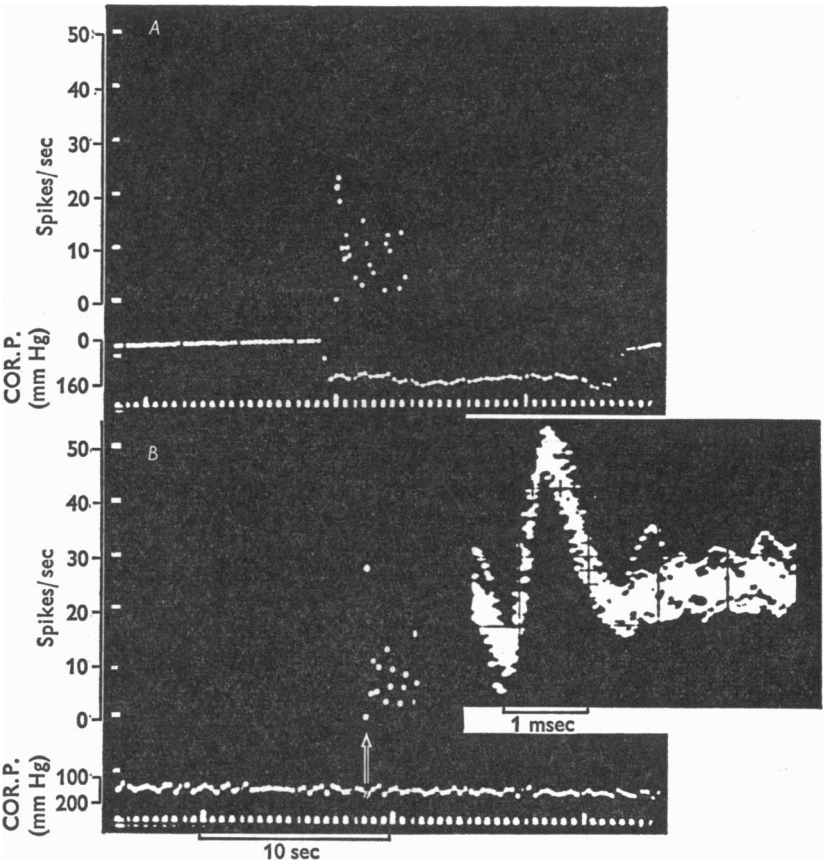

Fig. 3. *A*. Top: frequency histogram derived by the computer of the biphasic spike shown in Fig. 2 and in the inset of this Figure. Middle: coronary arterial pressure. In this particular sequence, the mean pressure rose from 28 to 148 mm Hg, whereas in Fig. 2 it rose from 0 to 160 mm Hg. The increase was shown as a downward deflexion. Bottom: time marks. Increasing inflow pressure provoked a burst in this unit which abated rapidly. Inset: each biphasic spike gave a point on the frequency histogram of *A*. All these spikes were superimposed for the inset.

B. Effect of manipulating the tip of the coronary cannula (at the arrow) on the same unit shown in 3*A* and the inset.

marked reflex increase in activity in the same unit (Fig. 6*C*). The increase occurred before a fall in aortic blood pressure appeared. This was important since systemic hypotension, no matter what the cause, elicited an increase in discharge thereby complicating interpretation (for the possible role

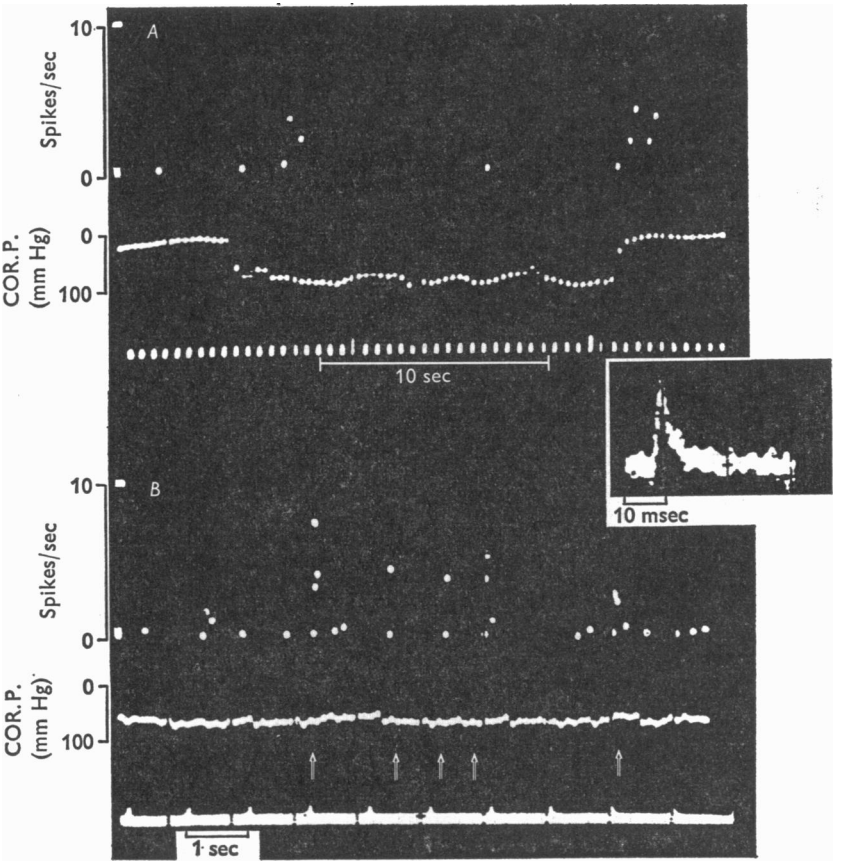

Fig. 4. *A*. Effect of increasing mean coronary arterial pressure from 12 to 75 mm Hg on an efferent unit in the third left ramus. Notation as in Fig. 3. Histogram plotted by computer. The spikes were superimposed and shown in the inset as in Fig. 3. There was some background activity and a burst as the pressure was increased (shown as a downward deflexion) and a burst with the decrease.

B. Effect of manipulating the tip of the coronary cannula at the arrows.

played by spinal anoxia, see Alexander, 1945). Ischaemia uncomplicated by systemic hypotension or other evidence of heart failure such as increased left ventricular end-diastolic pressure provoked a reflex increase in discharge in three additional single fibres and four multifibre preparations

(Table 1), but neither increased flow nor coronary sinus occlusion were effective.

The intracoronary injection of 1 μg veratridine prepared according to Brown (1966) provoked a reflex burst in two fibres that were also excited by increased coronary flow. Similar doses injected intravenously or into the root of the aorta had no effect on these units.

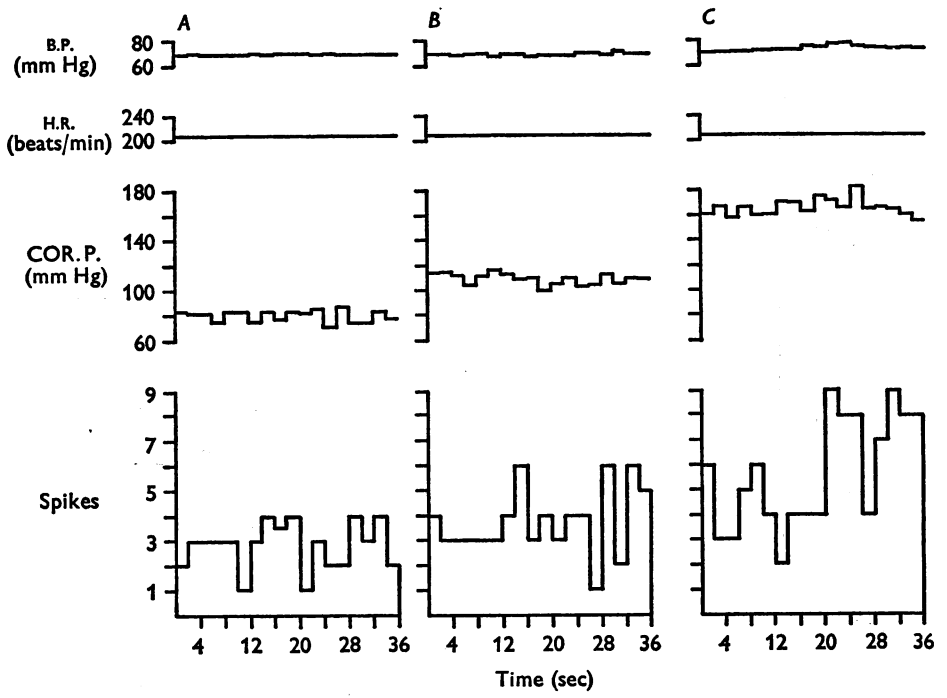

Fig. 5. Effect of increasing coronary arterial pressure on another efferent single unit from the third white ramus.

A. Control: flow of 4 ml./min. The discharge was counted per 2 sec and an active background was present.

B. Coronary flow was increased to 8 ml./min and mean inflow pressure rose from 80 to 110 mm Hg. The spike discharge increased and the increase in activity was sustained.

C. Flow increased to 20 ml./min and mean inflow pressure was now about 165 mm Hg. Again, the increased discharge was sustained.

Reflex reduction in sympathetic activity has not been observed using these procedures in vagotomized, spinal cats.

Reflex discharge in the inferior cardiac nerve. The reflex effects of increased coronary flow observed in preganglionic sympathetic fibres in spinal, vagotomized cats, have also been observed in three experiments using either slips dissected from the inferior cardiac nerve or the entire

nerve. Fig. 7*A* shows a multifibre preparation with very little background activity. Elevated coronary pressure elicited a large increase in discharge within 0·5 sec (Fig. 7*B*). Similar responses were evoked by occlusion of the coronary sinus (one experiment) and myocardial ischaemia (one experiment).

Effects of increased coronary inflow on afferent discharge in cardiac sympathetic nerves. The afferent fibre shown in Fig. 8*A* responded to an

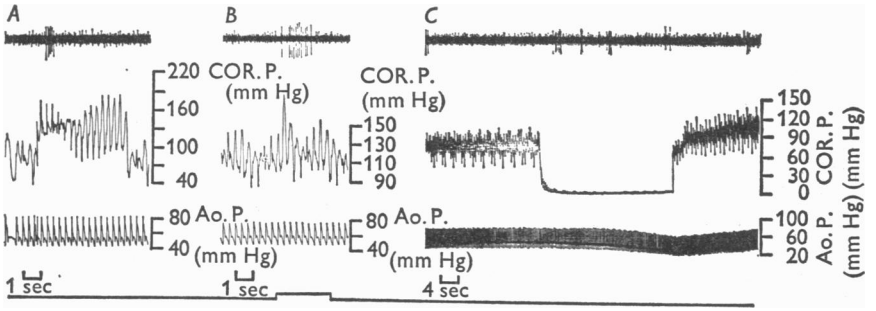

Fig. 6. *A*. Effect of increased coronary arterial pressure on an efferent unit in the third ramus. Same unit in *B* and *C*. Top or first trace: Spike discharge. Second: coronary arterial pressure. Third: aortic pressure.

B. Effect of coronary sinus occlusion during the signal. Brief increase of coronary arterial pressure occurred.

C. Effect of myocardial ischaemia. Note the decreased coronary resistance when ischaemia was terminated and control flow of 10 ml./min recommenced.

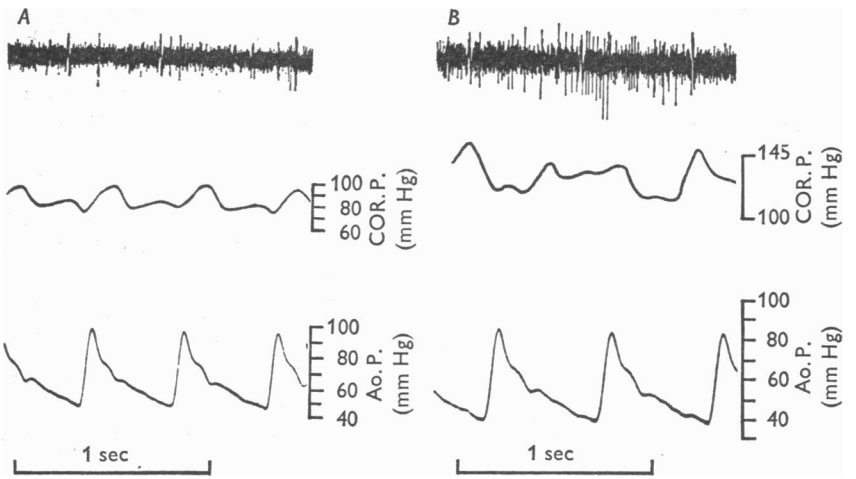

Fig. 7. Effect of increased coronary arterial pressure on the efferent discharge in a slip of nerve dissected from the inferior cardiac nerve in a vagotomized spinal cat. *A*. Control: mean coronary pressure 85 mm Hg. *B*. After 20 sec at new mean coronary pressure of 130 mm Hg.

increase in coronary arterial pressure after about 100 msec with a burst of impulses that decreased in frequency as the elevated pressure was maintained. Three additional single fibres showed similar short latency responses with adaptation.

Three other units responded to increased coronary inflow and pressure after a much longer latency (about 0·5 sec) with a short burst, followed by silence, despite the maintained pressure increase.

Finally, three single fibres which were spontaneously active, showed an increase in activity at higher pressures, which was sustained while the pressure increase was maintained.

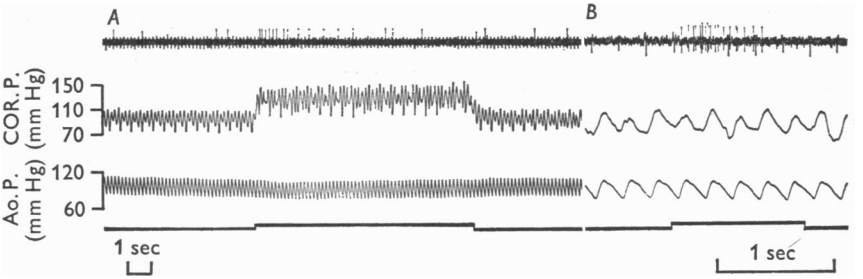

Fig. 8. *A*. Effect of increasing coronary arterial pressure from 90 to 130 mm Hg (mean values) on a single afferent fibre dissected from the inferior cardiac nerve. Pump flow increased from 6 to 10 ml./min. All records from spinal cats with both vagi sectioned, one or both common carotid arteries ligated. Top or first trace: electroneurogram. Second: coronary arterial pressure. Third: systemic arterial blood pressure. Fourth: reference trace and signal marker. A slight fall in systemic pressure began before and continued after the increase in coronary flow; the variation was phasic with positive pressure respiration.

B. Effect of manipulation of the tip of the coronary cannula during the signal, bottom trace. Pressure calibrations as in *A*; note faster time base.

All of the single units that responded to increased coronary arterial pressure were activated by moving the tip of the coronary cannula (Fig. 8*B*). In addition, five units were further localized by direct probing over the main left coronary artery and adjacent ventricle.

A total of ten single units and three multifibre preparations were stimulated by increased coronary arterial pressure. A summary of the number of single units and multifibre preparations activated by the different stimuli employed in these experiments is given in Table 2.

Afferent cardiac sympathetic fibres showed either an irregular low level background discharge or were quiescent. Only rarely (two experiments) did fibres show a discharge phasic with the pressure pulse. Spontaneously active fibres were neither silenced or showed decreased activity in response to the stimuli used.

Effects of coronary sinus occlusion on afferent activity. Occlusion of the coronary sinus excited four single fibres and four multifibre preparations. Two patterns of response were observed:

(*a*) low frequency burst of impulses (Fig. 9*A*) which in half of the cases decreased in frequency despite maintained occlusion (two single fibres,

TABLE 2. Number of afferent fibres excited by stimuli limited to the coronary bed

	Increased coronary arterial pressure	Coronary sinus occlusion	Myocardial ischaemia
Single units	10	4	8
Number of responses	46	14	17
Success rate (%)	100	100	94
Multifibre preparations	3	4	4
Number of responses	12	11	12
Success rate (%)	100	100	92

Two single units and two multifibre preparations included in column one are also entered in column two; these two single units are entered in column three as well. Three additional single units from column one are also in column three, making a total of five units excited by both increased coronary arterial pressure and myocardial ischaemia.

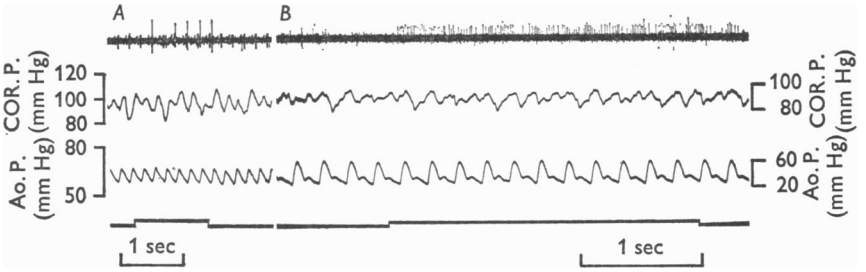

Fig. 9. *A*. Effect of coronary sinus occlusion during signal (bottom trace) on a single afferent fibre in the inferior cardiac nerve. A low frequency burst occurred. Coronary arterial pressure unchanged.

B. Another single afferent fibre showing high frequency burst of activity with coronary sinus occlusion (during signal). Discharge persisted throughout.

two multifibres). The peak frequencies attained during the burst in the single units were variable (1–10/sec).

(*b*) high frequency burst which decreased in frequency but was maintained throughout the occlusion (Fig. 9*B*) (two single fibres, two multifibres).

In 22/37 trials, sinus occlusion produced no change in inflow pressure. A rise of less than 10 mm Hg was observed in the remainder. Such increases of inflow pressure by themselves did not excite these fibres.

The fibres activated by coronary sinus occlusion were stimulated by intravenous injections of three to five ml. normal saline (three single fibres, three multifibre preparations) and by direct probing in the region of the coronary sinus and inferior vena cava (three single units, one multifibre preparation). Two single units and two multifibre preparations were also excited by increased coronary inflow.

Effect of an increase in coronary arterial flow combined with coronary sinus occlusion on afferent discharge. Two single fibres which were excited by

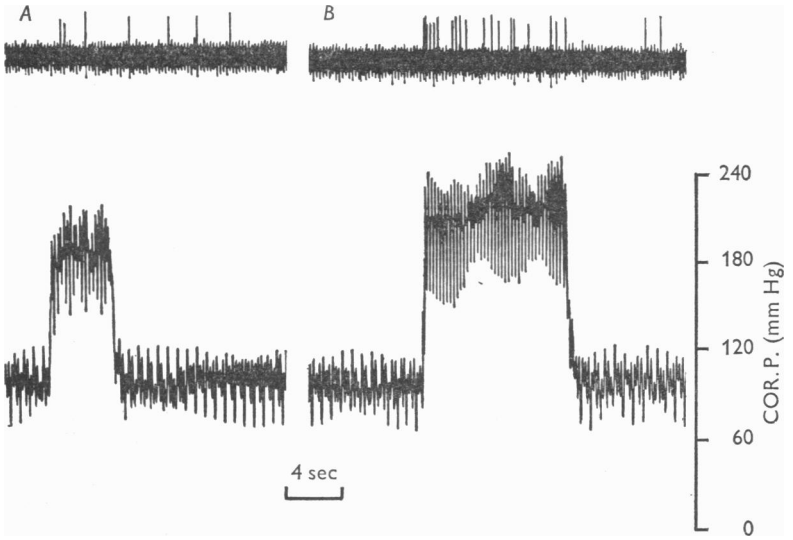

Fig. 10. *A*. Effect of increased coronary arterial pressure (coronary flow from 8 to 20 ml./min) on an afferent unit in the inferior cardiac nerve. Top: electroneurogram. Spikes retouched. Next: coronary arterial pressure.

B. Effect of increased inflow from 8 to 20 ml./min combined with coronary sinus occlusion. The inflow pressure in this case was 20 mm Hg greater than in *A*. The discharge was also greater.

increased coronary arterial flow were more greatly excited by a combination of increased flow and coronary sinus occlusion. When inflow was greatly increased, sinus occlusion always elicited an increase in inflow pressure. Thus, a small response to increased coronary arterial pressure became much greater when the coronary sinus was occluded (Fig. 10, 4/4 trials). In this case, occlusion provoked a large increase in coronary arterial pressure. Similar results were obtained from the other single unit (3/3 trials). At lower flows, coronary sinus occlusion had no effect on inflow pressure, or on the discharge of these two units.

Effects of myocardial ischaemia on afferent activity. Cessation of inflow always produced myocardial ischaemia within 10 sec (Brown, 1968).

Changes in aortic pressure, right and left ventricular pressures, right and left atrial pressures, and pulmonary arterial pressure followed after much longer intervals of 40 sec or more. Fig. 11 shows a single fibre that was spontaneously active and showed a marked increase in discharge within 10 sec of cessation of inflow. Computer analysis of another unit which was

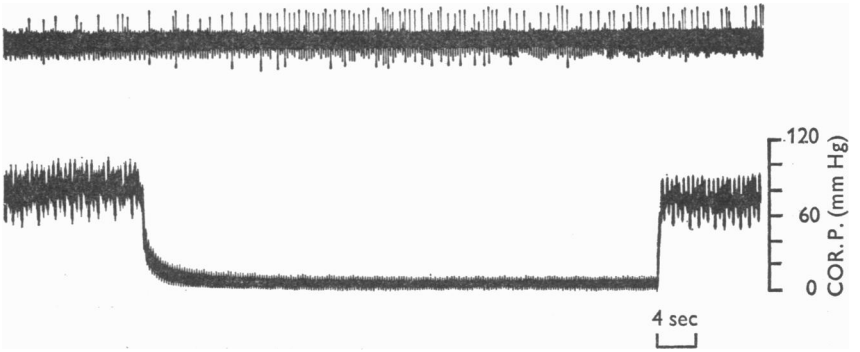

Fig. 11. Effect of myocardial ischaemia on an afferent unit in the inferior cardiac nerve. The sudden fall in coronary pressure occurred when the inflow was stopped. There was some background activity which showed a marked increase during ischaemia.

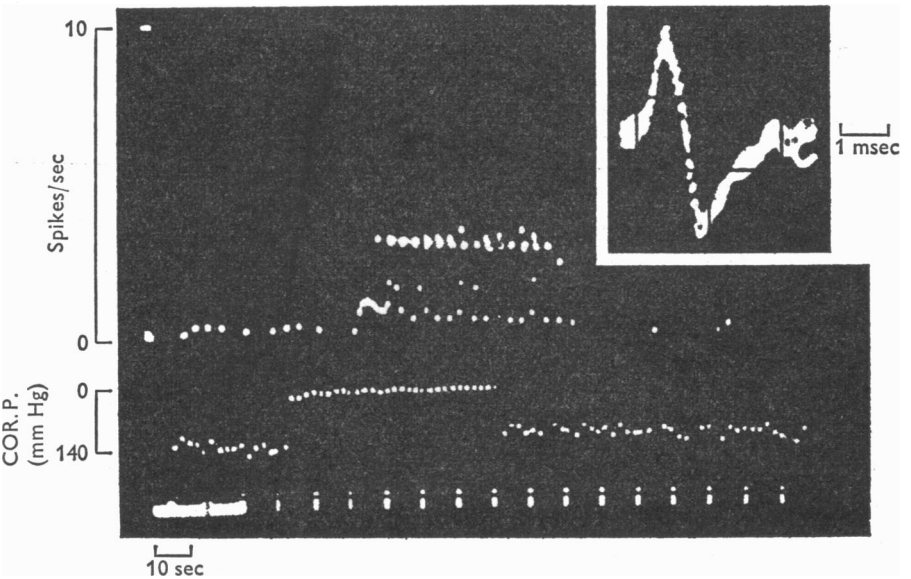

Fig. 12. Effect of ischaemia on a spontaneously active unit. Computer analysis. Fall in coronary pressure shown as an upward deflexion. Background discharge was markedly increased after 15–20 sec ischaemia. Note persistent after-discharge. Inset: each counted spike superimposed.

spontaneously active, is shown in Fig. 12. Twenty sec after the onset of ischaemia there was a marked increase in discharge. Fig. 13 shows a computer-derived frequency histogram of a fibre that was initially quiescent, and became activated after 15–18 sec of myocardial ischaemia. All fibres activated by ischaemia showed an afterdischarge which persisted for some 20–40 sec following the re-institution of coronary flow. The reduced coronary vascular resistance secondary to ischaemia persists for about the same length of time (Brown, 1968).

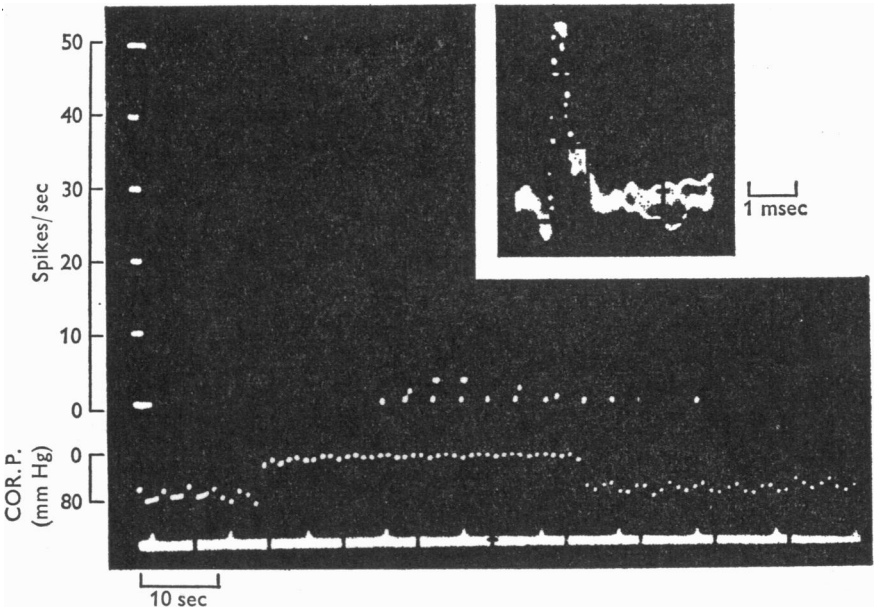

Fig. 13. Effect of myocardial ischaemia on an afferent unit from the third left ramus. Computer analysis. Fall in coronary pressure shown as an upward deflexion. No background activity was present. After 15–18 sec the discharge increased and persisted for some 20 sec after flow was recommenced.

A total of eight single fibres and four multifibre preparations were activated by ischaemia (Table 2). Three of the single fibres responded only to ischaemia. The other five were excited by increased coronary arterial pressure as well. Two of these fibres were also activated by coronary sinus occlusion and by the intracoronary injection of 0·5 ml. 10^{-2} HCl and 0·1 μg veratridine. Similar amounts of these agents injected into the right atrium or root of the aorta had no effect.

Location of afferent fibres in cardiac sympathetic nerves. Seventy per cent of the afferent recordings were made from slips of nerve dissected from the inferior cardiac nerve. Thirty per cent were made from the third left white

ramus. Each nerve contained fibres which showed the entire spectrum of responses described in this paper. While there may be some vagal contamination of the inferior cardiac nerve, it is extremely unlikely that any vagal fibres are present in the white rami.

DISCUSSION

Afferent fibres have been identified which were sensitive to increased coronary arterial pressure, myocardial ischaemia and coronary sinus occlusion. Some fibres were activated by one of these stimuli only, and others by all three stimuli and by veratridine and 10^{-2} M-HCl injected directly into the coronary artery. None were inhibited by the stimuli. We have indicated the number of fibres in each category including those excited by more than one stimulus, which were identified in the present experiments. It must be emphasized, however, that these numbers give no indication of the total number of afferent fibres that may be activated by one or more of the stimuli, and that the results of these experiments are considered to be entirely qualitative in this regard.

The fibres ran in the cardiac sympathetic nerves and were not aberrant vagal fibres since they were present in the white rami as well as the inferior cardiac nerve. The fibre size is unknown since conduction velocities were not measured. However, most, if not all, the afferent fibres in the inferior cardiac nerve have conduction velocities of 10–25 m/sec and belong to the Aδ category (Brown, 1967).

Most of the fibres were not influenced by the heart beat, although two fibres had a phasic rhythm in time with the pressure pulse. In this sense, they are quite different from the vagal coronary mechanoreceptors, all of which had a cardiac rhythm (Brown, 1965). The present fibres form the afferent limb of the excitatory coronary-sympathetic reflex also described in this paper.

The anatomical evidence for afferent fibres in cardiac sympathetic nerves originating from the heart was presented by Nettleship (1936) and Nonidez (1939). The fibres are connected to well defined bush or tree-shaped endings in the subendocardium, myocardium or adventitia of the coronary arteries but some terminate as naked endings in the interstitial space. Following section of the upper five thoracic dorsal root ganglia, they disappeared.

Location of the receptors. The receptors were in the heart since the stimuli used were restricted to the coronary circulation and nearby myocardium. Receptors responsive to increased coronary inflow alone were probably on the arterial side of the coronary bed upstream from the resistance vessels (see under *Nature of the stimuli*). Further evidence is that mani-

pulation of the tip of the coronary cannula or direct probing over the main left coronary artery excited these receptors. An explanation of the very long latency following increased pressure shown by some responsive receptors, may be related to changes in interstitial pressure which probably accompanied the elevated arterial pressure.

Receptors activated solely by coronary sinus occlusion were probably on the venous or capacitance side of the bed. Direct probing over the coronary sinus activated these fibres as well. Receptors activated by increased inflow or sinus occlusion may have been connected either to arteries or veins since the pressure–volume relations of the entire coronary bed would be changed by either manoeuvre. The location of the receptors excited by ischaemia alone, or by ischaemia as well as other stimuli, is unknown.

Nature of the stimuli. When coronary flow was increased, coronary pressure rose and either function may have been the provocative stimulus for responsive receptors. However, coronary sinus occlusion enhanced the response of receptors excited by increased inflow, inflow pressure rising further in each instance (7/7 trials). Thus, at a time when pressure was increasing, inflow was likely to be decreasing (the total coronary sinus flow in the cat comes from the left coronary artery; Brown, 1968). This strongly suggests that increased pressure rather than increased flow was the stimulus.

Receptors activated by coronary sinus occlusion alone were probably responding to changes in pressure or volume in the coronary veins since at normal flow inflow pressure was not altered significantly. Unfortunately coronary venous pressure was not measured; nor was the degree, if any, of ischaemia ascertained. It may not be surprising that increased coronary flow would, by producing changes in venous pressure or volume, stimulate such receptors.

Of those receptors excited by ischaemia, a few were stimulated by ischaemia alone but the remainder could also be excited by increased coronary arterial pressure, coronary sinus occlusion, direct probing of the heart, and the intracoronary injection of veratridine or 10^{-2} N-HCl. In terms of physiological stimuli, however, only the increased coronary arterial pressure and coronary sinus pressure would be operative, although in pathological conditions such as coronary artery disease, the ischaemia receptors could be excited.

The nature of the precise stimulus that activated receptors during myocardial ischaemia remains unknown (Brown, 1967). It is possible that some dilatation of the heart chambers occurs within 10 sec of the onset of ischaemia before any pressure changes occur, and it may be this mechanical stimulus that excites receptors during ischaemia. Against this is the fact that aortic occlusion or large increases in circulatory volume have little

effect on these receptors (Brown, 1967). A more likely explanation is that first offered by Lewis, Pickering & Rothschild (1930) to account for the pain of intermittent claudication, namely, that some substance or substances are released by ischaemic muscle that excite certain nerve fibres that transmit the impulses signalling the pain of ischaemia.

Characteristics of the reflex response to increased coronary arterial pressure or coronary sinus occlusion. These are summarized as follows:

1. Efferent fibres were reflexly excited by stimuli restricted to the coronary bed.

2. The response is abolished by sectioning the afferent fibres running through the stellate ganglia.

3. Afferent fibres display patterns very similar to those shown by the efferent fibres.

The stimuli required to evoke the reflex were substantial; this was probably due to persistent spinal shock. Other contributing factors might have been the extensive surgery, circulating catecholamines and compensated acid–base disturbances (Millar & Morris, 1961). Much smaller stimuli were required to excite the afferent fibres of the reflex.

Malliani, Pagani, Recordati & Schwartz (1970) have reported that aortic occlusion which would increase coronary arterial pressure caused either a reflex increase or decrease in sympathetic activity in spinal cats. It seems likely that circulatory receptors located elsewhere than the coronary bed can, when excited, produce a reflex decrease of sympathetic discharge in spinal animals.

Convergence of input (increased coronary arterial pressure, myocardial ischaemia and coronary sinus occlusion) onto the same unit was suggested in five experiments (see Fig. 6). However, the input might have come from the same afferent fibre since some afferents can also be excited by more than one of these stimuli.

Relation to the coronary depressor reflex. Using intact, anaesthetized cats, Brown (1966) demonstrated that increased coronary arterial pressure elicited a depressor reflex with associated reflex reduction of sympathetic discharge in the inferior cardiac nerve. The afferent limb was in the vagi. In spinal, vagotomized animals, the same stimulus provokes reflex excitation of sympathetic fibres in this nerve. One possibility is that two completely different populations of efferent fibres were studied in the two sets of experiments and the two patterns of response are part of the same reflex. Another possibility is that the same population was sampled, but two completely different reflexes were evoked by the same stimulus. This would require either a common interneuronal pathway or a common preganglionic neuronal pool. Experiments designed to test these alternatives are under way.

The afferent limb for the depressor reflex would be in the vagi, whereas the afferent limb for the excitatory coronary-sympathetic reflex would be in the cardiac sympathetic nerves. It is unlikely that vagotomy itself qualitatively alters the nature of the reflex since it does not do so in the case of the carotid sinus reflex (Heymans & Neil, 1958; Ninomiya & Irisawa, 1969). The depressor reflex ordinarily seems to prevail (Brown, 1966) and Coote, Downman & Weber (1969) have recently reported that spinal sympathetic reflexes are inhibited by the brainstem. Thus, these excitatory spinal reflexes may represent the most elementary organization for circulatory reflexes, upon which supraspinal influences exert their effects (Malliani *et al.* 1970). The neurogenic hypertension that follows sino-aortic denervation may in part be accounted for by such reflexes.

Reflex response to myocardial ischaemia. The evidence that the response to ischaemia is reflex, is similar to that adduced previously for the response to increased coronary pressure or occlusion of the coronary sinus. The response was elicited before any evidence of heart failure such as increased left ventricular end-diastolic pressure or decreased aortic pressure appeared. Thus, it seems unlikely that spinal anoxia (Alexander, 1945) or receptors located elsewhere than the heart, might be responsible.

Malliani *et al.* (1969) found that occlusion of a branch of the main coronary artery produced a reflex increase in sympathetic discharge. However, in a very few instances, a decrease in sympathetic activity was recorded. The discrepancy with the present experiments in which only excitatory effects were observed may be accounted for by the different procedures used to elicit myocardial ischaemia.

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